

## **Validating the Novel Method of Measuring Cortisol Levels in Cetacean Skin by use of an ACTH Challenge in Bottlenose Dolphins**

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### **LONG-TERM GOALS**

Our research group, under PI Thea Bechshøft, has recently shown that it is possible to extract and measure cortisol in cetacean skin (Bechshoft et al. 2015). The goal of the current project is to validate this novel method by measuring baseline cortisol levels in cetacean skin and demonstrating the correlation between an acute stress exposure and the ensuing skin cortisol response.

### **OBJECTIVES**

At the conclusion of the project period, we expect to have provided a greater understanding of a) the relationship in cetaceans between blood cortisol levels and skin cortisol levels, b) the period of time it takes before an acute stress response is measurable in the cetacean skin matrix, and c) cetacean baseline cortisol levels in skin, as well as inter- and intra-individual fluctuations.

### **APPROACH**

The project is executed under PI Dr. Thea Bechshoft, in collaboration with Dr. Dorian Houser (National Marine Mammal Foundation, USA), Dr. Bjarne Styrishave (Copenhagen University, Denmark), and Dr. Andrew J. Wright (George Mason University, USA and Aarhus University, Denmark).

The project will validate the recently developed skin cortisol analysis method (Bechshoft et al. Submitted) using skin samples collected from bottlenose dolphins (*Tursiops truncatus*). The dolphins will be sampled as part of an ongoing out-of water stress test and stress hormones study conducted by Dr. Dorian Houser in collaboration with the U.S. Navy Marine Mammal Program (MMP) under ONR project N000141110436. The stress test in brief: Each dolphin is asked to perform a routine, voluntary beach into a padded beaching tray. Immediately following beaching, a needle is inserted into the peduncle of the dolphin and blood is collected every 15 minutes for two hours, in addition to other manipulations (e.g. blubber biopsies). This process has shown to significantly raise both cortisol and aldosterone above baseline conditions and thus equals an acute stress response. The stress test will be

followed by sampling from various matrices over a longer period of time. For the skin cortisol validation project, non-invasive skin samples will be collected from a total of five animals at three points in time: pre-stress test, at time of the stress test, and post-stress test. The results of the stress test will determine the link between the level of cortisol in the blood and the skin of bottlenose dolphins. The results will also establish the delay between a spike in blood cortisol levels and any corresponding spike in skin cortisol levels, thus helping to validate the use of skin cortisol to assess chronic stress levels in bottlenose dolphins and other cetaceans. All skin samples will be analyzed for cortisol levels at the University of Copenhagen, Denmark (Bechshoft et al. 2015).

The project described above is closely related to another recently awarded project “Measuring and validating levels of steroid hormones in the skin of bottlenose dolphins (*Tursiops truncatus*)” (Award Number: N00014-15-1-2187), which expands on the range of hormones to be measured in the skin samples. Please see the FY15 report for the latter for further details.

## **WORK COMPLETED**

All skin sampling, including post-stress test collection, has been successfully completed. The resulting skin samples have been shipped to the University of Copenhagen, where the hormone analyses are currently ongoing.

## **RESULTS**

Hormone analyses are currently ongoing. Results are expected by February 1<sup>st</sup>, 2016, after which statistical data analysis will ensue.

## **IMPACT/APPLICATIONS**

A validated method for assessing cortisol in non-invasively collected cetacean skin samples will bring new possibilities for stress assessment in cetaceans, opening up a new avenue of research in physiological response studies following exposure to stressors. The current study will provide the validation of this novel method (Bechshoft et al. 2015) by measuring basal level cortisol in cetacean skin and demonstrating the correlation between an acute stress exposure and the ensuing skin cortisol response.

## **REFERENCES**

Bechshoft T, Wright A, Weisser JJ, Teilmann J, Dietz R, Hansen M, Björklund E & Styrishave B. Developing a new research tool for use in free-ranging cetaceans: recovering cortisol from harbor porpoise skin. *Conservation Physiology*, 3: doi:10.1093/conphys/cov016

## **PUBLICATIONS**

Bechshøft TØ, Wright AJ, Teilmann J, Dietz R, Hansen M, Weisser JJ & Styrishave B. Developing a novel stress research tool for free-ranging cetaceans: recovering cortisol from harbour porpoise skin. Talk given at the 20th Biennial Conference on the Biology of Marine Mammals Dunedin, New Zealand, 9-13 December 2013.

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